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Methods recommended (1) for the carbohydrate analysis of honey are little changed from those used 50 years ago. They are highly empirical and based upon insufficient knowledge of the sugars present in honey. In a recent study (2) of the determination of glucose and fructose, the authors examined five procedures and concluded that variance due to methods was as great as that due to differences among various floral types of honey. Thus, little confidence can be placed in comparison of results of analyses of honey by different existing methods. Täufel and Reiss (3) have concluded from a paper-chromatographic study of honey that the customary methods of analysis are inadequate.

This paper describes the use of carbon column chromatography as a pre-treatment for analysis, and presents analytical methods for determination of glucose, fructose, sucrose, reducing disaccharides, and higher sugars in the eluates. By separating honey into monosaccharide, disaccharide, and higher saccharide fractions before analysis, results are obtained that are more nearly related to the actual composition of the mixture. Because of the complexity of honey, however, some degree of empiricism still remains in the analytical procedure.

The procedure developed by Whistler and Durso (4) for separation of sugar mixtures into mono-, di-, tri-, and higher saccharides has been widely used for preparative work. In this method, the sugar mixture is adsorbed on a charcoal-celite column and successively eluted with water, 5 per cent ethanol, 15 per cent ethanol, etc., to separate the saccharides of increasing degree of complexity.

The quantitative aspects of this separation have not been neglected. McDonald and Perry (5) used carbon column adsorption to separate corn sirup into glucose, maltose, and dextrin fractions for analysis. They can separate a sample into these fractions in about one and one-half hours, using up to 50 pounds of pressure on the column. Eluate fractions are concentrated from 900 to 100 ml for analysis, which is done by conventional methods. They reported average recoveries of 96.5 per cent for glucose and 97.1 per cent for maltose from known mixtures.

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Alm (6) has recently described the use of carbon as the adsorbent in his gradient elution analyses of Schardinger dextrin hydrolyzates. The procedure as outlined does not appear suited for routine use on a number of samples, and if it were scaled up to provide sufficient material for fructose-glucose analysis of the monosaccharide fraction, excessive volumes and time would be required.

We have adjusted the column size, sample size, and eluant composition so that the time required for separation is reasonable, and little evaporation of fractions is required before analysis. This has required a study of the elution curves of honey for selection of the optimum eluant and adaptation of analytical methods to direct analysis of the effluent fractions.

The procedure described here has been applied to some 19 floral types of honey. Results show that considerably less glucose is present than heretofore realized, and reducing disaccharides are general components of all samples analyzed, as suggested by van Voorst (7) and Hurd, *et al.* (8). These results will appear in another publication.[1]

EXPERIMENTAL

Selection and preparation of column.—Since honey is largely a monosaccharide sirup, adjustment of sample size to weight of adsorbent was based largely on the disaccharide content. The adsorbent weight must be adequate to hold all disaccharide and higher material (estimated at below 100 mg for 1 g of average honey) whereas the preponderant monosaccharides pass through the column essentially unadsorbed. This adjustment to the disaccharide content permits the use of smaller columns (and hence more rapid flow and smaller eluant volumes) than if the column size were adjusted to the amount of monosaccharide likely to be present.

The columns were made from 22 mm (O.D.) Pyrex tubing, packed dry by the procedure of McDonald and Perry (5). After a glass wool plug is inserted and wetted, sufficient 1:1 mixture (by weight) of Darco G-60[2] and Celite 545 is added without tapping or suction to fill the column to a depth of 23.5 cm (about 20 g is required). Then suction is applied to the column outlet while the column is tapped gently with a cork ring. The depth of the packed portion should then be about 17 cm. After the upper part of the column is cleaned of carbon, a layer of filter aid is added, about 7–10 mm thick after gentle packing. The column is washed with 750 ml of water and next with 250 ml of 50% ethanol; and then is allowed to stand overnight under 50% ethanol before use. Washing with 250 ml water suffices to remove alcohol from the columns. The columns should not be permitted to run dry. Applying air pressure of 10 pounds should give flow rates with water of 6.3–7.7 ml per minute.

To find eluants suitable for quantitative recovery of honey sugars as mono-, di-, and higher saccharides in volumes convenient for analysis, three 1 g samples of clover honey were adsorbed, and then eluted with water, 1% ethanol, and 2% ethanol, respectively. With water, at least 350 ml was required for complete recovery, but with 1% ethanol, recovery of monosaccharides was complete with less than 250 ml. When 2% ethanol was used, disaccharide elution began before mono-

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[1] See p. 478.

[2] Mention of trade names in this paper does not imply endorsement or recommendation by the United States Department of Agriculture over similar products not mentioned.

saccharide elution was complete. Hence, 1% ethanol was selected, with 250 ml as the required volume for elution.

For disaccharide elution, three 1 g samples were adsorbed as above and washed with 400 ml of water, then with 5, 7, and 8% ethanol, respectively. Collection of 250 ml of 7% ethanol was selected for quantitative recovery, although 200 ml of 8% ethanol would have served as well. For elution of remaining carbohydrate material remaining on the column, 100 ml of 50% ethanol was used without further investigation.

Variability of carbon.—During this work, several lots of carbon (Darco G-60) were used. Considerable differences were noted in flow rate and in adsorbing capacity. Maltose saturation (determined by carrying 0.5 g maltose through the procedure and determining the maltose in the 7% ethanol fraction) was 180 and 301 mg for two samples. The former column showed poor disaccharide recoveries after 8 uses. When possible, selection should be made for high flow rate (6–8 ml/mm) and high maltose saturation. One lot of the carbon–filter aid mixture had a rate of 3.5 ml/min., which lengthened the procedure unduly.

During the routine analyses of honey samples, it was noted that, though occasional recovery experiments with pure disaccharides indicated that the columns were functioning satisfactorily, runs with half-size samples of honey would give higher results for disaccharides. There appears to be a progressive deterioration in the column performance when honey samples are analyzed. The cause for this has not been determined. It has been satisfactory to limit the number of uses of a column to about 8 samples and replace it at that time.

Adsorption procedure.—The column is washed with 230–250 ml of water to remove the 50% alcohol under which it is stored. Then 20 ml of 1% ethanol is passed through. Air pressure is disconnected, and the sample, dissolved in 10 ml of 1% ethanol, is quantitatively transferred to the column and washed successively as described below. Filtrates are collected in volumetric flasks. The eluate collected during the introduction of the sample is included in the 250 ml of the "*A*" fraction. After the "*C*" fraction is collected, the column may be stored under the 50% ethanol without further treatment. About 2 to 3 hours is required for the adsorption separation.

When the procedure outlined above was applied to honey (1.0 g samples), the eluates contained the following amounts of sugars:

(*A*) 1% ethanol: 300–500 mg fructose and 200–400 mg glucose in 250 ml.

(*B*) 7% ethanol: 3–60 mg sucrose and 40–100 mg reducing disaccharide in 250 ml.

(*C*) 50% ethanol: 5–50 mg reducing sugar, after hydrolysis, in 100 ml.

Determination of individual sugars in carbon column eluates.—Methods were modified when necessary to allow determination of these sugars without concentrating the solutions. In the work described here, the sugars used were as follows:

Glucose—Bureau of Standards, Standard Sample 41, lot 4006. Less than 0.05 per cent moisture.

Fructose—$[\alpha]_D^{25} = +92.8°$, moisture 0.31 per cent.

Sucrose—Commercial table sugar was used, containing less than 0.04 per cent moisture.

Maltose—Eastman maltose hydrate was recrystallized from aqueous

ethanol. Moisture on drying *in vacuo* by procedure of Cleland and Fetzer (9) = 6.74%; $[\alpha]_D^{25} = 131.6°$ on hydrate basis; 138.7° anhydrous.

FRACTION "*A*"

Determination of fructose.—The direct determination of fructose in mixture with glucose has several advantages over its calculation by difference between total reducing sugars and glucose (10). We used the procedure of Marshall and Norman (10), with slight modification. In this method, glucose is oxidized by hypoiodite, and residual fructose is determined by copper reduction. The amounts of sugar specified for their procedure (40–80 mg. in 20 ml) are higher than those occurring in the solutions from the carbon columns. For this reason and because of our use of a Shaffer–Somogyi reagent[3] different from that used by Marshall and Norman, we calibrated the procedure for fructose rather than use the Marshall–Norman equation.

Solutions containing fructose and glucose as shown in Table 1 were subjected to the following procedure. Results are shown in the table.

TABLE 1.—*Shaffer-Somogyi titrations of residual fructose after hypoiodite oxidation of mixtures of glucose and fructose at 18°C.*

SUGARS IN SOLUTION OXIDIZED		FRUCTOSE IN SOLUTION ANALYZED	0.005 *N* THIOSULFATE REQUIRED
GLUCOSE	FRUCTOSE		
mg	*mg*	*mg*	*ml*
8.00	8.10	0.202	1.01
19.9	20.2	0.504	3.59
18.1	21.5	0.537	3.85
28.8	30.5	0.762	6.00
40.0	40.4	1.010	8.23
37.6	41.0	1.025	8.32
55.7	64.1	1.602	13.72
80.0	80.8	2.020	17.56

The sugars were dissolved in 20.0 ml of 1% ethanol in a 200 ml volumetric flask. After the addition of 40.0 ml of 0.05 *N* iodine solution and the slow addition (with shaking) of 25.0 ml 0.10 *N* sodium hydroxide, the flask was immersed in the 18° (±0.1°) bath. After 10 minutes, the reaction was stopped by the addition of 5.0 ml *N* sulfuric acid, the flask was removed from the bath, and the excess iodine was reduced with fresh 1% sodium sulfite, with 2 drops of starch indicator. The solution was then neutralized to bromocresol green with *N* sodium hydroxide and made to volume with water. Reducing value was determined in duplicate on 5 ml aliquots with the Shaffer-Somogyi reagent noted previously.

A straight line was fitted to the data in Table 1 using the values from 3.59 to

[3] The Shaffer-Somogyi reagent 50 (11) with 5 g KI and 250 ml 0.10 *N* potassium iodate/l was used because of greater stability.

17.56 ml thiosulfate. The equation, calculated from the line, for the determination of fructose by this procedure was

$$a = 0.1090b + 0.113 \quad \text{(I)}$$

where a = mg fructose in 5 ml aliquot between 0.50 and 1.75 mg fructose;
b = ml 0.00500 N thiosulfate.
Average deviation of the experimental values from the line was 0.37%.

$$500\,a = \text{Mg fructose in 250 ml 1\% ethanol column eluate} \quad \text{(II)}$$

Determination of glucose.—A recent study (2) of the determination of glucose and fructose in honey demonstrated the value of the hypoiodite oxidation method for glucose in comparison with indirect determination by difference between total reducing sugars and fructose determined polarimetrically or by oxidation. Significantly greater precision was found for the Lothrop–Holmes (12) procedure for glucose than for other methods studied. Marshall and Norman (10) have modified this method slightly, principally by specifying oxidation at 16–18°C. rather than 20°. They also used direct determination of fructose after hypoiodite destruction of glucose, rather than determination by difference, as recommended by Lothrop and Holmes.

The factors previously cited set the glucose concentration in the carbon column filtrate at about 250–450 mg in 250 ml, or 20–36 mg of glucose in a 20 ml aliquot. This sugar concentration is considerably lower than that used by Lothrop and Holmes (60–80 mg) or Marshall and Norman (40–80 mg).

Preliminary glucose oxidations by the Lothrop–Holmes procedure were carried out in the 20° (±0.1°) bath. Table 2 shows the results. This apparent increasing over-oxidation with decreasing glucose concentration was noted by Marshall and Norman (10).

TABLE 2.—*Oxidation of glucose at 20°C. by hypoiodite*

GLUCOSE OXIDIZED	0.05 N THIOSULFATE REQUIRED	GLUCOSE[a] FOUND	RECOVERY	MG GLUCOSE PER ML THIOSULFATE
mg	*ml*	*mg*	*per cent*	
16.6	3.96	17.8	107.1	4.202
23.9	5.62	25.3	105.8	4.253
35.8	8.20	36.9	103.1	4.366
39.3	8.94	40.2	101.1	4.396

[a] Theoretical factor of 4.502 mg glucose/ml thiosulfate was used.

A series of oxidations was carried out in which volume, iodine and alkali concentration, and extent of acidification were varied, but this error was not reduced. Marshall and Norman reported that for 50 mg glucose, recovery of 101.3 per cent at 20.5° was reduced to 99.6 per cent by using an

18° oxidation bath.[4] Table 3 shows the effect of oxidation in an 18° (±0.1°) bath upon recoveries.

TABLE 3.—*Oxidation of glucose at 18°C. by hypoiodite*

GLUCOSE OXIDIZED	0.05 *N* THIOSULFATE REQUIRED	GLUCOSE[a] FOUND	RECOVERY	MG GLUCOSE PER ML THIOSULFATE
mg	*ml*	*mg*	*per cent*	
12.20	2.75	12.38	101.47	4.436
24.40	5.44	24.49	100.37	4.485
20.56	4.58	20.62	100.29	4.489
41.12	9.06	40.79	99.19	4.539
				Av. 4.487

[a] Ml×4.502.

The conversion factor in the last column of Table 3 shows the same trend as for the oxidation in the 20° bath, though the spread is less. The average factor found, 4.487, is 99.67 per cent of theoretical and compares with the 4.484 value used by Marshall and Norman. The latter will be used here.

When mixtures of glucose and fructose are subjected to hypoiodite oxidation, some oxidation of fructose takes place. Lothrop and Holmes corrected the calculated glucose value by subtracting 1.3 per cent of the fructose content, an experimentally derived correction for the oxidation of fructose in the presence of glucose by alkaline iodine solutions. Although Marshall and Norman concluded that a constant correction of this type is not satisfactory for all concentrations, they used a constant correction for their range (40–80 mg. glucose and 40–80 mg fructose).

Various amounts of glucose and fructose were oxidized under the same conditions as in Table 3 (20 ml sugar solution in a cork-stoppered 250 ml Erlenmeyer flask, 20 ml 0.05 *N* iodine, 25 ml 0.1 *N* NaOH added over 30 seconds, placed in water bath at temp. 18±.1°; 5 ml 2 *N* H_2SO_4 added after 10.0 minutes and titrated with 0.05 *N* thiosulfate). Ratios of fructose to glucose were 1 and 1.5, the normal limits found in honey. Table 4 shows the results.

The factor from the last column in Table 4 was plotted against weight of fructose. The line

$$c = 0.0219 - 0.0002F \qquad \text{(III)}$$

was found to fit adequately between the limits 10 and 70 mg fructose where

c = ml 0.05 *N* thiosulfate per ml fructose and

F = mg fructose in the 20 ml.

[4] Analyses of the cooling curves of samples (initial temperature 26.5°) in the 20° and 18° baths for 10 minutes showed that the average temperature was 22.5° and 20.1°, respectively. Lothrop and Holmes specified oxidation "at 20°" (12) without stating whether this referred to the bath temperature, air temperature, or temperature of the solution.

TABLE 4.—*Oxidation of glucose at 18°C. by hypoiodite in the presence of fructose*

GLUCOSE	FRUCTOSE	TITER, 0.05 *N*	MG GLUCOSE ÷ 4.484	COL. 3–COL. 4	ML 0.05 *N* THIO PER MG FRUCTOSE
mg	*mg*	*ml*		*ml*	
14.6	15.3	3.54	3.26	0.28	0.018
25.4	23.3	6.08	5.66	0.42	0.018
31.7	31.8	7.53	7.07	0.46	0.015
41.1	39.2	9.61	9.16	0.45	0.012
14.0	22.8	3.53	3.12	0.41	0.018
24.3	37.4	6.01	5.42	0.59	0.016
31.5	47.6	7.68	7.02	0.66	0.014
39.6	60.9	9.54	8.83	0.71	0.012
40.9	44.0	9.61	9.12	0.49	0.011
39.8	59.6	9.61	8.88	0.73	0.012
20.3	31.1	5.07	4.53	0.54	0.017
21.4	20.9	5.16	4.77	0.39	0.019

From these data, the following equation for the iodometric determination of glucose in the presence of fructose under these conditions was derived:

G = factor (ml 0.05 N thiosulfate − correction for fructose oxidation), or

$$G = 4.484\left(\text{ml 0.05 } N \text{ thiosulfate} - F \times \frac{\text{ml thiosulfate}}{\text{mg fructose}}\right). \qquad \text{(IV)}$$

Since

$$\frac{\text{ml 0.05 } N \text{ thiosulfate}}{\text{mg fructose}} = 0.0219 - 0.0002F,$$

$$G = 4.484\ [\text{ml 0.05 } N \text{ thiosulfate} - F\ (0.0219 - 0.0002F)\ ], \text{ or}$$

$$G = 4.484\ (\text{ml 0.05 } N \text{ thiosulfate} - 0.0219F + 0.0002F^2), \qquad \text{(V)}$$

where

G = mg glucose in the 20 ml oxidized, between the limits 10–50 mg glucose and 10–65 mg fructose.

12.5 G = mg glucose in the 250 ml 1% ethanol eluate (VI)

For substitution in equation (V), F may be found from equation I by multiplying a by 40.

Table 5 shows glucose recovery for the mixtures in Table 4, calculated by this equation. From the data in the last column, a variance of 0.11 mg and a standard deviation of 0.33 mg may be calculated.

In applying this procedure to the analyses of column eluates, it was found that the 1 per cent alcohol caused erratic results due to the iodoform reaction. Since correction by blank was not successful, it was necessary to remove the alcohol by evaporation before analysis. Therefore, 20 ml aliquots of the "*A*" fraction (1 per cent alcohol) in the 250 ml Erlenmeyer flasks used for the determination were evaporated to dryness on the steam bath in a current of air. Twenty ml of water was added before analysis.

Table 5.—Recovery of glucose in mixture with fructose by hypoiodite oxidation

GLUCOSE	FRUCTOSE	TITER, 0.05 *N*	GLUCOSE[a] FOUND	RECOVERY	ERROR
mg	*mg*	*ml*	*mg*	*per cent*	*mg*
14.6	15.3	3.54	14.6	100.0	0.0
25.4	23.3	6.08	25.5	100.4	+0.1
31.7	31.8	7.53	31.6	99.7	−0.1
41.1	39.2	9.61	40.6	98.8	−0.5
14.0	22.8	3.53	14.0	100.0	0.0
24.3	37.4	6.01	24.5	100.8	+0.2
31.5	47.6	7.68	31.8	100.9	+0.3
39.6	60.9	9.54	40.1	101.3	+0.5
40.9	44.0	9.61	40.5	99.0	−0.4
39.8	59.6	9.61	40.4	101.5	+0.6
20.3	31.1	5.07	20.5	101.0	+0.2
21.4	20.9	5.16	21.5	100.5	+0.1

[a] By equation V.

FRACTION "B"

Determination of reducing disaccharides as maltose.—Erratic values for maltose calibration in preliminary work indicated that a longer period of heating in the Shaffer–Somogyi oxidation might be necessary. It was found that thirty minutes in the boiling water bath was necessary to reach a constant titer in this determination.[5] Solutions (5 ml) of maltose in 7 per cent ethanol containing the amounts of anhydrous maltose shown in Table 6 were treated with the Shaffer–Somogyi reagent (as used for fructose) for thirty minutes in the boiling water bath. The titers are shown in Table 6.

TABLE 6.—*Determination of maltose in 7% ethanol with Shaffer-Somogyi reagent*[a]

ANHYDROUS MALTOSE PER 5 ML	0.005 *N* THIOSULFATE REQUIRED
mg	*ml*
0.183	0.57
0.456	1.61
0.913	3.56
1.460	6.15
1.826	7.67
3.652	15.81

[a] SS 50, 5 g KI and 250 ml 0.1 *N* iodate/l; 30 min. heating.

[5] During the course of the work it was demonstrated statistically that blank determinations using water, 1% or 7% alcohol, and 15 or 30 min. heating times all give the same titration value in the Shaffer-Somogyi determination; hence special blanks need not be run.

From these data the following relationship was obtained:

$$M = 0.2264e + 0.075 \quad \text{(VII)}$$

where

M = mg anhydrous maltose in 5 ml

e = ml 0.005 N thiosulfate.

50 M = milligrams anhydrous maltose in 250 ml 7% ethanol eluate. (VIII)

Determination of sucrose.—The 7 per cent ethanol eluate from the carbon column contains sucrose in addition to a considerable proportion of reducing disaccharide. To determine the sucrose, the change in total reducing value after mild acid hydrolysis was used. The procedure devised is outlined below. The hydrolysis is essentially that of the A.O.A.C. (1) for sucrose.

To a 5.00 ml sample in 7% ethanol in a 10 ml volumetric flask is added 1.00 ml dilute HCl (sp. gr. 1.1029) and 1 ml water. It is immersed in a water bath at 60° ±1°C. for 12 minutes and cooled to room temperature. The solution is made just alkaline to bromocresol green with 5 N NaOH and immediately brought to the acid side with 2 N H_2SO_4. It is then made to volume, and the reducing value of 5.00 ml is determined by the Shaffer-Somogyi procedure; 15 minute heating is used. Table 7 shows a calibration of the procedure with the weight of sucrose shown.

TABLE 7.—*Shaffer-Somogyi titration of hydrolyzed sucrose solutions*

SUCROSE IN 5 ML HYDROLYZED	0.005 N THIOSULFATE REQUIRED
mg	*ml*
0.510	1.75
1.004	3.95
2.008	8.72
2.510	11.28

To determine sucrose in the carbon column eluate, 5 ml aliquots are subjected to the procedure described above, and the sucrose equivalent is read from a curve constructed from the data in Table 7. From this value is subtracted the sucrose equivalent (from the curve) of the free reducing sugars in the solution. This may be obtained from the maltose titer. To avoid an extra determination of free reducing value with a 15 minute heating period, the maltose titer (30 min. heating) is multiplied by 0.92 (determined experimentally) and then used. The difference is then the milligrams of sucrose in 5 ml of the column eluate.

$$50(S_1 - S_2) = \text{mg sucrose in 250 ml 7\% ethanol eluate} \quad \text{(IX)}$$

where

S_1 = mg sucrose equivalent to sucrose titer

S_2 = mg sucrose equivalent to 0.92 × maltose titer (e in equation VII)

FRACTION "C"

Determination of higher sugars.—The 50 per cent ethanol eluate from the carbon column should contain all carbohydrate material from trisac-

charide to at least the heptasaccharide (13). To obtain an estimate of this fraction, it was hydrolyzed by the procedure which von Fellenberg (14) applied to honey dextrin—hydrolysis at 100°C. in 1 *N* sulfuric acid for 45 minutes.

To 25 ml of the 50% ethanol eluate in a 50 ml volumetric flask were added 5 ml of 6 *N* HCl and 5 ml of water. After heating by immersion in a boiling water bath for 45 minutes, the flask was cooled, neutralized to bromocresol green with 5 *N* sodium hydroxide, and made to volume; the reducing power was determined on 5 ml by the Shaffer–Somogyi reagent. Glucose equivalent may be obtained from published values (11).

$$\text{Milligrams of higher sugars in 100 ml 50\% ethanol eluate} = 40 \times \text{mg glucose found.} \quad \text{(X)}$$

ANALYSIS OF KNOWN MIXTURES

Mixtures of glucose, fructose, sucrose, and maltose in the approximate proportions found in honey were dissolved in 10 ml 1 per cent ethanol, placed on the carbon columns, and analyzed by the procedures outlined above. Table 8 shows the results. Average recovery of maltose and sucrose

TABLE 8.—*Analysis of known sugar mixtures*

MIXTURE NO.	GLUCOSE			FRUCTOSE			MALTOSE			SUCROSE		
	PRESENT	FOUND		PRESENT	FOUND		PRESENT	FOUND		PRESENT	FOUND	
	mg	*mg*	*per cent*	*mg*	*mg*	*per cent*	*mg*	*mg*	*per cent*	*mg*	*mg*	*per cent*
1	301.0	302.6	100.54	325.0	320.5	98.62	68.6	68.6	100.0	52.8	50.8	96.5
2	299.6	304.5	101.63	330.7	327.5	99.03	63.9	63.5	99.4	52.8	52.4	99.3
3	320.0	317.0	99.06	364.5	360.0	98.77	95.1	92.6	97.4	55.2	53.3	96.6
4	319.1	320.4	100.41	386.0	384.5	99.61	82.8	81.8	98.8	62.5	59.6	95.4
5	306.0	310.1	101.35	416.1	414.0	99.49	75.3	73.6	97.7	50.4	47.5	94.3
6	324.0	323.2	99.77	385.6	382.0	99.77	75.4	73.4	97.2	52.0	49.7	95.6
Av.			100.46			99.21			98.4			96.3

was of the same order as that found by McDonald and Perry (5). As expected, average recovery of monosaccharides was better and within the limits of the analytical methods, since adsorption is not so much of a factor under these conditions.

To determine whether materials present in honey have any effect upon the retention of the disaccharides on the carbon columns, weighed amounts of various sugars were added to four 0.8 g samples of a clover honey. These were adsorbed, eluted, and analyzed as already described. Table 9 shows the results.

In the table are shown three analytical values for each sugar as percentage of the honey; the value for the sample to which the sugar in question was added is shown in milligrams only. The average of these three values

TABLE 9.—*Analyses of mixtures of honey and added sugars*

HONEY	SUGAR ADDED		GLUCOSE		FRUCTOSE		SUCROSE		MALTOSE		HIGHER SUGARS	
grams	*Kind*	*mg*	*mg*	*per cent*	*mg*	*per cent*	*mg*	*per cent*	*mg*	*per cent*	*mg*	*per cent*
0.7644	Glucose	105.3	361.1	——	287.5	37.61	10.50	1.37	47.1	6.16	6.56	0.86
0.7867	Fructose	100.2	265.5	33.75	396.0	——	12.10	1.54	48.9	6.22	6.84	0.87
0.8085	Maltose	75.0	268.2	33.17	304.0	37.60	12.85	1.59	123.3	——	10.60	1.31
0.7735	Sucrose	60.5	259.7	33.57	296.5	38.33	68.15	——	48.7	6.30	7.00	0.90
Av.				33.50		37.85		1.50		6.23		

for each sugar was used to calculate the original amount of each sugar in the honey samples to which that sugar was added (Table 10). The difference between the values (sugar calculated in honey+sugar added) −(sugar found) is shown in Table 10. Here again, recoveries of the disaccharides are of the same order of magnitude as those in Table 8 for the pure sugar mixtures. Recovery calculated on the total amount of each sugar present is also shown in Table 10.

TABLE 10.—*Analytical recovery of sugars in honey-sugar mixtures*

	GLUCOSE	FRUCTOSE	SUCROSE	MALTOSE
Wt honey sample, g	0.7644	0.7867	0.7735	0.8085
Sugar in honey (Table 9), %	33.50	37.85	1.50	6.23
Wt sugar added, mg	105.3	100.2	60.5	75.0
Total sugar in sample, mg	361.4	398.0	72.1	125.3
Total sugar found, mg	361.1	396.0	68.2	123.3
Difference, mg	0.3	2.0	3.9	2.0
Recovery (% total sugar)	99.92	99.50	94.4	98.40

SUMMARY

A carbon column adsorption procedure was used for separation of the sugars of honey into monosaccharides, disaccharides, and higher sugars. Integrated with suitable analytical methods, the procedure permitted determination of the sugars of honey with greater accuracy than previously attained. A more realistic picture of the carbohydrate composition of honey was thus obtained.

Recoveries of glucose, fructose, maltose, and sucrose from mixtures averaged 100.46, 99.21, 98.42, and 96.28 per cent, respectively; recoveries of these sugars when added to honey were 99.92, 99.50, 98.40, and 94.40 per cent of the total individual sugar present.

REFERENCES

(1) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D.C., 1950, pp. 518–521.
(2) WHITE, J. W., JR., RICCIUTI, C., and MAHER, J., *This Journal*, **35**, 859 (1952).
(3) TÄUFEL, K., and REISS, R., *Z. Lebensm.-Untersuch. u.-Forsch.*, **94**, 1 (1952).
(4) WHISTLER, R. L., and DURSO, D. F., *J. Am. Chem. Soc.*, **72**, 677 (1950).
(5) MCDONALD, E. J., and PERRY, R. E., JR., *J. Research Natl. Bur. Standards*, **47**, 363 (1951).
(6) ALM, R. S., *Acta Chem. Scand.*, **6**, 1186 (1952).
(7) VOORST, F. T., v. *Chem. Weekblad*, **38**, 538 (1941).
(8) HURD, C. D., ENGLIS, D. T., BONNER, W. A., and ROGERS, M. A., *J. Am. Chem. Soc.*, **66**, 2015 (1944).
(9) CLELAND, J. E., and FETZER, W. R., *Ind. Eng. Chem., Anal. Ed.*, **14**, 27 (1942).

(10) MARSHALL, C. R., and NORMAN, A. G., *Analyst*, **63**, 315 (1938).
(11) SHAFFER, P. A., and SOMOGYI, M., *J. Biol. Chem.*, **100**, 695 (1933).
(12) LOTHROP, R. E., and HOLMES, R. L., *Ind. Eng. Chem., Anal. Ed.*, **3**, 334 (1931).
(13) WHELAN, W. J., BAILEY, J. M., and ROBERTS, P. J. P., *J. Chem. Soc.*, (1953), 1293.
(14) FELLENBERG, T. v., and RUFFY, J., *Mitt. Gebiete Lebensm. Hyg.*, **24**, 367 (1933).